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Project Summary

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Determination of TCDD in Industrial and Municipal Wastewaters

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This program was undertaken in an effort to develop an analytical method for the determination of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) in industrial and municipal wastewaters. The method includes the extraction of TCDD with methylene chloride, clean up with columns and quantitative determination with gas chromatography (electron capture detector) and gas chromatography/mass spectrometry (GC/MS). The detection limit was found to be 0.003 μg/L.

In conjunction with the development of an analytical method, the stability of TCDD in organic solvents and chlorinated water samples was also studied. It was found that TCDD solutions prepared in benzene, acetone and methanol remained stable during both cold storage (4° C) and at room temperature (25° C). However, degradation of TCDD in water was observed as a result of chlorination followed by prolonged storage.

This Project Summary was developed by EPA's Environmental Monitoring and Support Laboratory, Cincinnati, OH, to announce key findings of the research project that is fully documented in a separate report of the same title (see Project Report ordering information at back).

Introduction

Under provisions of the Clean Water Act, the Environmental Protection Agency is required to promulgate guidelines establishing test procedures for the analysis of pollutants. The Clean Water Act Amendments of 1977 emphasize the control of toxic pollutants and declare the 65 priority pollutants and classes of pollutants to be toxic under Section 307(a) of the Act. This report is one of a series that investigates the analytical behavior of selected priority pollutants and suggests a suitable test procedure for their measurement.

The purpose of this study was to develop and evaluate several techniques for the analysis of 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) by gas chromatography (GC) and gas chromatography-mass spectrometry (GC/MS). In conjunction with the development of the analytical method, studies to determine the optimum conditions for water samples storage were also conducted by evaluating the effects of pH and residual chlorine. The stability of TCDD in organic solvents such as benzene, acetone and methanol was also studied to determine the shelf life of both the standard and the extract solutions.

While the focus of this study was to develop an analytical method and to conduct related studies for the 2,3,7 8-TCDD, the technique and results could apply to other isomers and analogs.

Method Development

Extraction

The purpose of this experiment was to assess the effect of pH of the water sample on extraction efficiency. Two extraction solvent systems commonly used in pesticide analysis were used to extract TCDD from water at pH 2, 7, and 10. The

solvents tested were methylene chloride and the solvent mixture 15% methylene chloride in hexane.

The water sample was prepared by placing one liter of steam-distilled water in a 2.0-liter separatory funnel. The desired pH level was obtained by addition of the necessary amount of either 0.1 N HCl or 0.1 N NaOH. After pH adjustment, 100 ng TCDD dissolved in 100 μL methanol was added to the water. This was then briefly shaken. Then 60 mL of the respective solvent was added, and the sample shaken vigorously for approximately one minute.

This was repeated twice using 60 mL solvent. These extracts were then combined and passed through 50 gm hexane-washed Na_2SO_4 into a Kuderna-Danish (K-D) apparatus and placed on a water bath. The sample was concentrated to approximately 10 mL, at which time. 1.0 mL toluene was introduced. This was further concentrated to about 5 mL. The concentrate was transferred to a 10 mL graduated test tube and concentrated to about 1 mL with a gentle stream of nitrogen at room temperature. Two portions of 5 mL of hexane were added to exchange for the methylene chloride by taking the solvent down to 1 mL after each addition with nitrogen. The final volume of the extract was adjusted to 1.0 mL. Each data point represents results from duplicate injections of the analyses as compared to the response of a standard of TCDD in hexane prepared on the day of analysis.

Both solvent systems were proven effective in extracting TCDD from all pH levels, and recoveries in the 90% + range were consistently obtained. Pure methylene chloride is recommended in the protocol because the chance of emulsion formation is less than when a mixture of methylene chloride in hexane is used. It is also the solvent used in extracting neutral compounds and pesticides in a number of other EPA procedures; therefore, these extracts can be used for TCDD determination.

Since there was no significant difference in recovery of TCDD at different pH levels of the water, it is not necessary to adjust the pH of the sample before extraction.

Since TCDD is a trace impurity in 2,4,5-trichlorophenoxy acetic acid (2,4,5-T) it often occurs as a neutral compound in minute quantity together with much higher concentrations of acidic chlorinated compounds, so it is desirable to remove the acidic compounds from the extract by washing the organic extract with a dilute solution (1 N) of sodium hydroxide or potassium hydroxide. Such a technique was well documented to be successful in many methods especially those dealing with formulations of 2,4,5-T, and it was adopted in this study.

Column Chromatographic Clean Up

Silica Gel Column

The silica gel column method was adapted from the procedure developed by Dow Chemical Co. in analyzing TCDD in 2,4,5-T acid formulations. A glass column (30 cm x 1 cm i.d.) was packed with 14 gm silica gel (Curtin Matheson Scientific Inc., Davidson 923 brand, 100-200 mesh, high purity grade) and equilibrated with 20% benzene in hexane. After the addition of either sample extract or TCDD standard, 20% benzene in hexane mixture was added to the column as necessary to maintain liquid above the silica bed, until 40 mL were collected. The eluate was condensed and analyzed for TCDD by GC/EC and/or GC/MS. This column is particularly useful in cleaning water samples obtained from effluents of manufacturing plants which formulate herbicides such as 2,4,5-T. The acidic compounds are related by this column. TCDD can be eluted using a mixture of 20% benzene in hexane. Triplicate studies in our laboratory have shown that adding 100 ng of TCDD to this column enables a mean recovery of 90%, with a standard deviation of 8%.

Alumina Column

A 15 cm x 1 cm i.d. column was packed with neutral alumina (Fisher Scientific Co., Fisher A540, neutral, activated at 130°C for 24 hours). The sample was introduced after the column was developed with 50 mL hexane, and TCDD was eluted with the following solvent systems:

1) 50 mL 3% methylene chloride in hexane
2) 50 mL 20% methylene chloride in hexane

The 20% methylene chloride in hexane portion was analyzed for TCDD by GC/EC and/or GC/MS.

Tests which include eluting 100 ng TCDD through this column show that TCDD is recovered from the 20% methylene chloride in hexane with a mean recovery of 86% (standard deviation 9%) from three runs. No TCDD was detected in the 3% methylene chloride mixture or in the pure methylene chloride eluate. This column is routinely used in our laboratory to clean up hexachlorophene samples and to separate polychlorinated biphenyls from TCDD. These procedures have proved to be effective with wastewater samples originating from manufacturers of 2,4,5-trichlorophenol, hexachlorophene and related products.

Charcoal-silica Gel Column

Many evaluations of columns packed with activated coconut charcoal were performed. However, due to the strong absorption of TCDD in charcoal, recoveries of TCDD were poor. This was true even with solvents such as methanol, acetone, ethyl acetate, methylene chloride and benzene. In an attempt to reduce the amount of charcoal in this column, silica gel was added as a dilutent.

The column consisted of a disposable Pipette (Fisher Scientific Co., Cat., No. 13-678-6A, 5 mm i.d.) packed to a length of 5 cm with a mixture of one part activated coconut charcoal (Fisher Scientific Co. 5-690-A. 50-200 mesh) to 140 parts of silica gel (Curtin Matheson 100-200 mesh, Davidson 923, high purity grade). It was eluted in the following manner:

1) Equilibrate the column with 5 mL of hexane.
2) Transfer extract into the column with hexane (any benzene or methylene chloride in the extract must be exchanged into hexane prior to addition to the column).
3) Elute with 10 mL of hexane, discard.
4) Elute with 10 mL of benzene.
5) Concentrate the benzene fraction carefully for analysis.

Results were obtained by adding to the charcoal silica gel column 10 ng and 100 ng of TCDD and eluting as mentioned above. Recoveries from six replicate runs are given in the following table:

Table 1. *Recoveries Of TCDD From Charcoal/Silica Gel Column*

TCDD Added	*Mean Recovery*	*Std. Dev.*
10 ng	*46*	*3.6*
100 ng	*43*	*5.4*

The above data indicated no significant difference in the percentage of recovery at two spiking levels, and despite the somewhat poor recoveries the standard deviations were excellent.

Gas Chromatography

This laboratory has experienced both clean samples that could be analyzed with an electron capture detector (ECD) and packed columns and very contaminated samples that could only be GC/MS coupled with capillary columns. Although it is more convenient to use the computerized multiple-ion-detection mode of the GC/MS system, the Programmable Multiple Ion Monitor (PROMIN) was also found to be very satisfactory. The following is a discussion on the various gas chromatographic columns used on this project.

Packed Columns

1) 1.5% OV-17 + 1.9% QF-1 on Gas Chrom Q (or 1.5% SP-2250 + 1.95% SP-2401 on Supelcoport), 100/120 mesh, 108 cm, 4 mm i.d., glass.
2) 3% OV-17 on Gas Chrom Q, 100/120 mesh, 180 cm, 2 mm i.d., glass.
3) 1% SP-2250 on Suplecoport, 120 cm, 2 mm i.d. glass.

While all three packed columns performed equally well, column 1 is recommended because it is the same column frequently used by the U.S. Environmental Protection Agency in the analysis for pesticides and has been successfully used routinely in our laboratory.

Capillary Columns

1) SP-2250, 30 meters, 0.25 mm i.d., 0.80 o.d. glass.
2) SP-2100, 30 meters, 0.25 mm i.d., 0.80 mm o.d. glass.

Again, both columns performed well, but the SP-2250 is recommended because it separated 1,2,3,4-TCDD and 2,3,7,8-TCDD isomers better than SP-2100 column. When 1,2,3,4-TCDD is used as the internal standard, the SP-2250 capillary column must be used.

Detection Limit

The instrumental detection limits were determined by measuring five times the signal to noise ratio for both ECD and GC/MS. **Table 2 summarizes the instruments and column used, the operational conditions and instrumental sensitivities.**

Internal Standards

In addition to the normal usages of internal standards for quantitative computation, a surrogate compound to be added to the water, which is less toxic than the hazardous 2,3,7,8-TCDD, is useful to varify the efficiency of the entire analytical method. For GC/MS determination, $C1^{37}$-TCDD and C^{13}-TCDD are the most common internal standards that are added to the extract. The former has the advantage of yielding a single peak (m/e=328) with good intensity for the MID mode while the latter gives a cluster of isotopic peaks for better confirmation.

When an ECD is used, compounds other than labeled ones must be used as internal standards. When 1,2,3,4-TCDD was tried, it was found that it behaved very similarly to the 2,3,7,8-TCDD. The only drawback with the 1,2,3,4-TCDD is that it elutes very close to 2,3,7,8-TCDD from packed gas chromatographic columns, and capillary columns must be employed to separate the two isomers. Some other TCDD isomers can be better separated than 1,2,3,4-TCDD from 2,3,7,8-TCDD, but they are generally not available commercially and typically not pure enough to be used.

Table 2. Instrumental Detection Limits

Instrument	Column	Splitting Ratios	Sensitivity
GC/ECD[1]	Packed[3]	—	10-20pg
	Capillary[4]	1/50 to 1/75	10-20-pg
GC/MS[2]	Packed[3]	—	10-20 pg
	Capillary[4]	Splitless	10-20 pg

[1] *Varian Model 3700 equipped with Nickol 63 detector.*
[2] *Finnigan Model 4000 GC/MS system coupled with INCOS computer, operated on the Multiple Ion Detection (MID)/Mass Fragmentography mode for ions 320, 322 and 324.*
[3] *Suplecoport 100/120 mesh coated with 1.5% SP-2250 + 1.95% SP-2401 packed in a 180 cm x 2 mm i.d. glass column. Column temperature was 220°C. Carrier gas for GC/ECD was 5% methane + 95% Argon at 25 mL/min, and helium at 25 mL/min for GC/MS.*
[4] *SP-2250 coated on a 3 m x 0.25 mm i.d. glass column (Supelco No. 20-3714 or equivalent) with helium as carrier gas at 30 cm/sec linear velocity. Column temperature was 210°C.*

The Recommended Protocol

Based on the findings mentioned in the above sections, a protocol was formulated to analyze TCDD in industrial wastewaters. Figure 1 is a flow chart of these procedures, and a detailed protocol is attached to the full report. The protocol includes three clean up columns investigated during the course of this study. The order of employing each of the columns as indicated in the flow chart is the same order which our laboratory follows in analyzing our wastewater samples and the analyst should experiment with the particular type of wastewater before choosing the number and type of columns. Since some loss of TCDD is almost unavoidable during sample manipulation, it is recommended that the analyst reduce to a minimum the number of clean up steps.

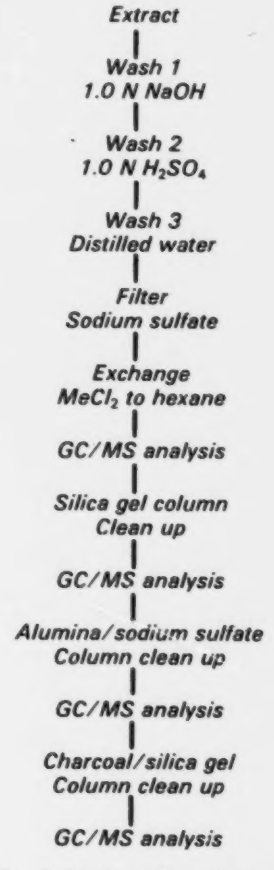

Figure 1. *Laboratory flow chart of protocol for processing wastewater extracts*

In order to determine the precision and accuracy of the protocol submitted with this report, five water samples were spiked with 100 ppt of 2,3,7,8-TCDD and then carried through the entire protocol. Analyses were performed after extraction as well as after passage through each of three columns. The results obtained are shown in Table 3.

In addition to recoveries performed on distilled water, seven industrial wastewater samples were also spiked with various amounts of 2,3,7,8-TCDD and then analyzed. The spiking levels and results are as follows:

The above data indicated the four samples which were cleaned up with the silica gel column only yielded slightly lower percentages of recovery than the spiked distilled water, while the recoveries of the last three samples were very close to that of spiked distilled water after going through all three clean up columns. Taking the experimental errors, the differences between levels of spiking as well as the qualities of the wastewater into consideration, the recoveries between clean and industrial waters are actually rather close.

Two sets of seven municipal wastewater samples, each obtained from the Sacramento Wastewater Treatment plant, (See Table 4), were spiked with TCDD at levels of 0.015 μg/L, or 5 x detection limit and 0.15 μg/L, 50 x detection limit and analyzed for precision and accuracy studies according to the recommended protocol. Preliminary studies indicated that the alumina column clean up was required before the sample can be analyzed by GC/MS. At 0.015 μg/L, 83.7 ± 5.1% recovery was obtained. At 0.15 μg/L, the recovery was 82.2 ± 4.2%.

Preservation Studies

Since chlorine, a very powerful oxidizing agent, is the most common disinfectant used in water treatment, its effect on the level of TCDD was investigated. A preservation study was conducted to determine the changes, if any, of TCDD in water with and without chlorine treatment at three pH levels. The results of this study can serve the purpose in determining the optimum sampling and storage of water samples as well as how soon such samples must be extracted after sampling.

Procedure

Water samples of 1 liter in a gallon-bottle at pH levels of 2, 7, and 10 were spiked with 1 ppb of TCDD together with 10 ppm chlorine (from sodium hypochlorite) and stored at temperatures of 4°C and 25°°C for 14 days. A similar set of samples without chlorine spiked with 1 ppb of TCDD served as controls. The water was extracted and analyzed by GC/MS, because sodium hypochlorite causes interference with GC/EC determinations.

Table 3. *% Of Recoveries Of Spiked Water Samples*

	After Extraction	1 Column[a] Clean up	2 Columns[b] Clean up	3 Columns[c] Clean up
Mean	93.	85	77	38
Standard Deviation	2.6	2.3	12	8.2

[a] *Silica gel*
[b] *Silica gel + alumina*
[c] *Silica gel + alumina + mixture of charcoal and silica gel.*

Table 4. *Recoveries Of TCDD From Wastewater*

Sample Indentification[1]	Spiking Level, ppb	# of Clean Up Columns	Instrument	% Recovery
1	20	One	GC/MS	63
2	40	One	GC/MS	70
3	80	One	GC/MS	75
4	20	One	GC/MS	71
5	100	Three	GC/EC	38
6	100	Three	GC/EC	51
7	100	Three	GC/EC	41

[1] *Industrial wastewater from other EPA projects, original source unknown. No TCDD was detected on any of the unspiked samples.*

Table 5. *% Recoveries Of 1 μg/L Of TCDD In Water After Chlorine Treatment*

	14 Days 4°C		14 Days 25°C	
pH Level	Mean	Std. Dev.	Mean	Std. Dev.
2	43.6	6.2	50.5	6.0
7	49.7	5.5	37.6	10.3
10	42.5	8.2	32.1	8.6

Table 6. *% Recoveries Of 1 μg/L Of TCDD In Water Without Chlorine Treatment*

	14 Days 4°C		14 Days 25°C	
pH Level	Mean	Std. Dev.	Mean	Std. Dev.
2	65.5	8.0	77.5	10.9
7	67.5	7.6	77.0	5.9
10	74.4	4.6	79.1	7.2

Results and Discussion

The following results were obtained (Tables 5 and 6). Each value represents the mean of triplicate runs.

Since the study was conducted only at a single storage period of 14 days, two temperatures and three pH levels, it is difficult to establish any trend and generalization from the above data. One conclusion that can be drawn is that the loss of 30% to 60% of TCDD due to chlorination followed by 14 days of storage even at a low temperature of 4°C, can cause significant discrepancies between the actual level of TCDD in the wastewater in the environment and the analytical results. In order to study further the rate of the disappearance of TCDD, a second experiment which was designed to eliminate potential loss due to transferring, extraction and evaporation in the normal analytical procedure was conducted. In this experiment, 90 ng TCDD in 9.0 μL acetone was spiked into 90 mL water contained in a 100-mL volumetric flask. It was then chlorinated with 10 ppm chlorine (from sodium hypochlorite), and 1.0 mL purified hexane was added to the solution to extract the TCDD at the end of the storage period. The hexane layer was then analyzed by GC/MS.

Storage periods of 0, 3, 6, 9, 12, 15 and 21 days were studied. The results obtained are given in Table 7. No degradation was observed in a control set which was held at identical experimental conditions except it was not chlorinated.

The degradation rate observed in this experiment is somewhat slower than those reported in the first. This is probably due to the modified procedure used in the second experiments to eliminate losses due to non-oxidative causes such as evaporation.

Table 7. The Effect Of Chlorination On TCDD Concentration In Water

Storage Period	pH 7 at 25°C % of TCDD Recovered	% of TCDD Loss
0	100	0
3	94.9	5.1
6	89.6	10.4
9	82.9	17.1
12	81.2	18.8
15	77.9	22.1
21	71.6	28.4

Conclusion and Recommendations

Based on the experimental part of this project, an analytical protocol was developed to determine the concentration of TCDD in wastewater. The recovery percentages and the standard deviations obtained from spiked samples were quite comparable to other published or accepted methods for TCDD analyses in various substrates, with the exception of the low recoveries whenever the charcoal-silica gel column was employed. The consistent performance as indicated by the small value of the standard deviation and clean up capability of the charcoal-silica gel column, made it possible to include it in the recommended protocol. Almost all methods for TCDD analysis call for the use of either Cl^{37}-TCDD or C^{13}-TCDD as internal standards. These labeled compounds, which must be analyzed by GC/MS, not only improve the accuracy in quantitation, but sometimes act as a carrier, especially during column chromatographic clean up, to improve recoveries. The use of labeled internal standards is also recommended for wastewater when charcoal-silical gel clean up is required.

One of the goals of this study was to develop an analytical method employing only single column chromatographic clean up followed by gas chromatographic determination with packed column and electron capture detector. Unfortunately, the common existence of other chlorinated compounds in high concentrations when trying to measure low levels of TCDD in wastewater samples made it necessary to recommend the use of sophisticated techniques and equipment such as the GC/MS and capillary columns. The original intent of this research effort was to develop a routine monitoring method which government and industrial laboratories would have the equipment and technical ability to follow. The protocol developed in this study can be considered a monitoring method, but may require relatively sophisticated laboratories and technique development.

Degradation of the TCDD in water was observed as a result of chlorination followed by storage. If the mechanism of the destruction of TCDD by chlorine is due to the oxidizing power of chlorine, then other oxidation processes such as ozonation in water treatment must be studied. The loss of TCDD in chlorinated water suggests that water samples from chlorinated effluents should be extracted and analyzed as soon as possible unless a non-reactive preservative can be found. It is recommended that additional studies be conducted to determine the shelf life of TCDD in both natural and wastewaters.

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***James E. Longbottom** is the EPA Project Officer (see below).*

The complete report, entitled "Determination of TCDD in Industrial and Municipal Wastewaters," (Order No. PB 82-196 882; Cost: $9.00, subject to change) will be available only from:

National Technical Information Service
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